CERTIFICATION

This is to certify that this project was carried out, complied and reported by DADA, IFEOLUWA MERCY with matriculation number 1501012005 under the supervision of Mrs. O.O Ayodele.

MRS. O.O AYODELE **PROJECT SUPERVISOR**	**DATE**
DR. A. A. ADEIGA **HOD**	**DATE**

DEDICATION

This report is dedicated to God Most High for His protection, love, wisdom and knowledge He provided me with throughout the compilation of my research project. Also to my loving parents for their undying love and care during the course of this project.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by varying or persistent hyperglycemia resulting from insufficient action of insulin. It is observed as the body's incapacity to efficiently regulate the sugar balance which leads to severe complications, pancreatic damage resulting in the dysfunction of alpha and beta cells causes disordered glucose homeostasis. This study intends to evaluate the effect of *Carica papaya* leaf methanol extract on plasma blood glucose, lipid profile, bleeding time and clotting time of STZ induced diabetic rats.

Thirty male Wistar rats were randomly divided into five groups and administered different doses of the extract for 14 days while normal control and diabetic control received distilled water. The plasma glucose concentration and lipid profile in the blood plasma of experimental animals were determined using Randox Kits; 20µl of samples and standard were mixed with 200µl of working reagent and incubated at 37°C for 10minutes. The absorbance was read at 500nm against a blank. Both the bleeding and clotting time were also determined as reported by Raof *et al.* (2013) and Ibu and Adeniyi (1989) respectively. *C. papaya* leaf methanol extract caused significant ($p<0.05$) reduction in plasma glucose concentration, plasma total cholesterol, triglycerides, VLDL-C, LDL-C, bleeding time and clotting time of the diabetic rats while plasma HDL-C was significantly elevated. The qualitative phytochemical screening showed the presence of steroids, anthraquinone, tannin, and other bioactive compounds. This study showed that the methanol extract of *C. papaya* exerted a hypoglycemic, hypolipidemic and procoagulatory effect which improves the lipid profile in diabetic rats.

Key words: Diabetes mellitus, *Carica papaya*, lipid profile

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Diabetes mellitus (DM) is a long-lasting disease that is comparatively common all over the world (Akbarzadeh, *et al.,* 2007). DM is caused by an unusual high concentration of sugar in the blood which might be a direct consequence of deficiency of insulin (Narkhede, *et al.,* 2011). It is a complicated disorder characterized by hyperglycemia causing malfunction in insulin secretion or insulin action. Diabetes mellitus is perhaps the world's prevailing increasing metabolic sicknesses affecting hundreds of millions and having occurrence rate of about 1 % in developed countries (Hauwa'u, *et al.,* 2014). Universal occurrence of diabetes has intensely continued to grow (Polonsky, 2012).

Diabetes mellitus is a potentially morbid condition characterized by hyperglycemia, and about eighty percent (80%) of people with diabetes mellitus die from thrombosis due to enhanced activation of platelets and clotting factors (Carr, *et al.,* 2015). That is, diabetic patients are prone to hypercoagulation. Dyslipidemia has been reported as an important risk factor that predisposes to cardiovascular disease in diabetes mellitus. This is because diabetic patients suffer from impaired lipid profile, which can lead to increased atherogenic index and coronary heart disease (Khan *et al.,* 2007).

Diabetic treatment (therapies) include, but not limited to, insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides and glinides. However, these agents are not without adverse effects (Ogundele, *et al.,* 2017). The World Health Organization has been particularly focused on the potential offered by herbal medicine, the main subfield of traditional medicine

practiced in different countries (WHO, 2012). Ethno-botanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes.

More than 1200 plant species are used worldwide in diabetes treatment, and experimental studies support the antihyperglycemic activity of large number of these plants (Yasmeen and Prabhu, 2012). Discovery of anti-hyperglycemic agents from plants with fewer side effects has been major focus of recent researches. Anti-hyperglycemic properties of the plants are in direct relationship with their ability to reinstate the function of pancreatic tissues by causing an increase in insulin output or inhibiting intestinal absorption of glucose or facilitating the metabolites in insulin-dependent processes (Patel, *et al.,* 2012). There have been quite a number of researches on different components of the *Carica papaya* Caricaceae family, and several bioactive compounds have been discovered in the plant (Sathasivam, *et al.,* 2009). *Carica papaya* leaves are traditionally used to treat diseases like malaria, dengue, and jaundice. They have been reported to possess immunomodulatory and antiviral activities (Yogiraj, *et al.,* 2014). Other diseases that have been reported to be controlled by *C.papaya* traditionally in Nigeria includes; abdominal discomfort, pain, malaria, diabetes, obesity, infections, and oral drug poisonings. Adeneye and Olagunju (2009) and Ahmad, *et al*. (2011) reported the therapeutic potential of *Carica papaya* on dengue and malaria, while Owoyele, *et al*. (2008) confirmed the anti-inflammatory potency of the plant. Aruoma, *et al.* (2010) reported the effect of *Carica papaya* fruits and leaves on the sugar reducing tendency of the plant. In view of these reports, it is essential to investigate the acclaimed anti-hyperglycemic effect of *C. papaya* leaves and the effect on lipid profile of diabetic rats.

1.2 Statement of problem

Diabetic mellitus is a pathological condition characterized by hyperglycemia and diabetic patients suffer from impaired lipid profile, which can lead to increased atherogenic index and coronary heart disease. Prevalence of DM is growing rapidly, rising to pandemic levels especially in the developing world. Quite a number of antidiabetic and hypolipidemic agents have been developed over the years, however, these agents are not without adverse effects. In recent years, traditional and corresponding medicine have been an improvement in its popularity for the treatment of different diseases as herbal drugs generally have low toxic effects. *C. papaya* fruits and leaves have been reported to possess antidiabetic activity (Aruoma, *et al.*, 2010). In this context, it is important to explore and confirm *C. papaya* leaves as a possible source of novel hypoglycemic and hypolipidemic drugs.

1.3 Aim

To investigate the effect of methanol extract of *C. papaya* leaves on lipid profile and glucose concentration in Streptozotocin (STZ) induced diabetic male Wistar rats.

1.4 Objectives of study

1. To test for the phytochemical components of *C. papaya* leaf methanol extract.
2. To determine the effect of *C. papaya* leaf methanol extract on blood glucose concentration of diabetic rats.
3. To determine the effect of *C. papaya* leaf methanol extract on plasma lipid profile of diabetic rats.
4. To determine the effect of C. papaya leaf methanol extract on blood clotting and bleeding times of diabetic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disease characterized by hyperglycemia along with impaired glucose metabolism and other factors that produce energy, such as lipids and proteins. This metabolic disorder is the consequence of insulin secretion deficiency or insulin action resistance or both (Ahmed, *et al.,* 2011). The failure of the body to control the sugar equilibrium efficiently results in serious complications such as hyperglycemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis, and death-causing coma. Several lipid defects are correlated with diabetes mellitus; emerging proof confirms the crucial position of hyperlipidemia, primarily high blood cholesterol, especially Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein (VLDL-C) in atherosclerosis-related illness growth (Andallu, *et al.,* 2009). There are significant defects in lipid metabolism and lipoproteins in diabetes, which in turn depend on the extent of insulin deficiency, insulin resistance, obesity, diet, and concomitant main and secondary causes of hyperlipidemia. A number of strange lipoproteins and other lipids occur in diabetic hyperlipidemia, and these interact with free radicals resulting in increased lipid peroxidation in plasma, tissue and membranes, and ultimately comprehensive harm to the tissue. Lipid peroxidation is well established source of free radicals that play a significant part in diabetes etiopathogenesis and its problems (Andallu, *et al.,* 2009).

Diabetes mellitus is a possibly morbid disease characterized by hyperglycemia and most individuals with diabetes mellitus die from thrombosis as a result of increased platelet activation and clotting factors (Carr, *et al.,* 2018). That is, hypercoagulation is susceptible to diabetic

patients. Hyperglycemia appears to cause coagulation abnormality in diabetic patients (Ceiello, 1993).

2.2 Classification of diabetes

2.2.1 Type 1 diabetes mellitus (TIDM)

T1DM is insulin-dependent and is distinguished by loss of insulin generation in beta cells of the pancreas Langerhans islets resulting in insulin deficiency (Kumar, *et al.,* 2013).

2.2.2 Type 2 diabetes mellitus (T2DM)

T2DM is not insulin-dependent and is characterized by resistance to insulin and insufficient insulin production leading to hyperglycemia. T2DM is the most common form of diabetes, representing more than 90% of all diabetic incidence (Kumar, *et al.,* 2015). Globally, 382 million people were diabetic in 2013, which is expected to reach 592 million by 2035, leading to an increase of 55 percent. Western Pacific individuals were at the top with 138.2 million diabetes in 2013, followed by South East Asia and Europe. Most people with diabetes come from low-and middle-income countries and between the ages of 40 and 59. India is second to China in the number of people with diabetes; in 2013, India had 65.1 million diabetes compared to China's 98.4 million. In many countries, diabetes and its complications are major causes of death. In 2013, 5.1 million fatalities were caused by diabetes mellitus and $548 billion was spent worldwide on diabetes treatment (Kumar, *et al.,* 2015).

2.2.3 Gestational diabetes mellitus (GDM)

Dysfunction of insulin receptors in GDM leads to elevated concentrations of blood glucose during pregnancy. The diabetes treatment with herbal formulations is preferred over other techniques due to low costs and less side impacts (Kumar, *et al.,* 2015). It has been shown that

traditional medicines are better than conventional medications in diabetes treatment (Kumar, *et al.,* 2015). During pregnancy, synthetic oral anti-diabetic agents are not safe to use and may cause serious side effects (Kumar, *et al.,* 2015).

2.3 Measurement of diabetes

It is normal to have blood sugar levels below 140 mg / dL (7.8 mmol / L). Diabetes is indicated by reading more than 200 mg / dL (11.1 mmol / L) after two hours. Prediabetes is indicated by reading between 140 and 199 mg / dL (7.8 mmol / L and 11.0 mmol / L).

2.4 Streptozotocin (stz)

Streptozotocin is an antimicrobial agent and was also used as an alkylating chemotherapy agent (Lenzen, 2008). STZ is a toxic compound of glucosamine-nitrosourea that causes damage to DNA cells. DNA damage induces the activation of Poly ADP Ribose Polymerase (PARP), which is probably more important for the induction of diabetes than the DNA damage itself (Szkudelski, 2008).

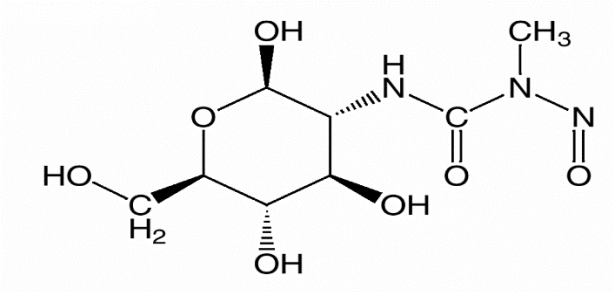

Figure 1: Chemical structure of STZ

2.5 Treatment of diabetes

For the therapy of diabetes, pharmaceutical drugs such as sulfonylureas and biguanides are either too costly or have undesirable side impacts or contraindications (Debidas, *et al.,* 2005). Metformin is a popular anti-diabetic medicine used for this research as the normal medication.

2.5.1 Metformin

Metformin is a biguanide with a strong base of 12.4 pKa and therefore predominantly exists as a protonated cation at physiological pH. Despite its hydrophilicity, metformin can be transferred via organic cation transporters (OCT) across cell membranes (Mustafa, *et al.,* 2015).

This drug's molecular action was not obviously created. However, the hepatic impacts can be ascribed to the main action of this medication. Hepatic sensitivity to insulin is increased thereby reducing gluconeogenesis and glycogenolysis which contributes to the post prandial plasma glucose-lowering effects (Shaw, *et al.,* 2005).

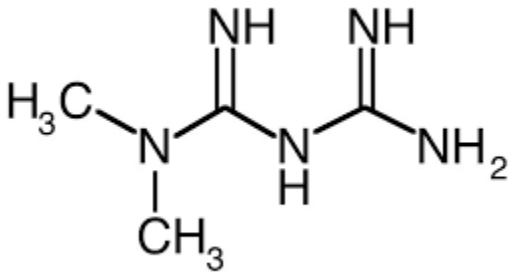

Figure 2: Chemical structure of Metformin

2.6 Lipid profile

2.6.1 Triglycerides (TAG)

Triglyceride is a glycerol-derived ester with three fatty acids. Triglycerides are the human body's major constituents of fats. They cannot freely pass through the cell membrane of the body, and unique enzymes current on blood vessel walls called lipoprotein lipase must break down triglycerides into three fatty acids and glycerol. Atherosclerosis is caused by high triglyceride levels (Mustafa, *et al.,* 2015).

2.6.2 Total cholesterol (TC)

It is a measure of LDL-C, HDL-C and other lipid components.

2.6.3 High density lipoprotein (HDL)

They are often called "healthy cholesterol." It is one of five lipoprotein groups that removes fat molecules from the cells. Increasing concentration of HDL is heavily correlated with reducing atherosclerosis accumulation within the artery wall. But studies have shown that the capacity to transport cholesterol bile is still missing in HDL mice. HDL mainly carries cholesterol to the liver that is then excreted into the bile and intestines (Mustafa, *et al.*, 2015).

2.6.4 Low density lipoprotein (LDL)

They assist carry fat molecules in the extracellular water around the body. LDL is a "poor cholesterol .The lipid profile does not measure LDL particles but it is estimated by using Friedewald, *et al.*, 1972 equation; Total cholesterol – VLDL-C – HDL-C.

2.6.5 Very low density lipoprotein (VLDL)

VLDL is produced by the liver and released into the bloodstream. They carry triglycerides. VLDL is similar to LDL cholesterol but LDL mainly carries cholesterol to the tissues instead of triglycerides (Mustafa, *et al.*, 2015).

2.7 Carica papaya (Linn.)

C. papaya is a family Caricaceae plant, also known as pawpaw with potential medicinal properties, cultivated in most tropical countries (Sudhakar and Vidhya, 2014). Papaya is a powerhouse with nutrients and is available throughout the year. It is a rich source of powerful threes of antioxidant vitamin C, vitamin A, and vitamin E; minerals, magnesium, and potassium; (Aravind, *et al.*, 2013).

Figure 3: Pawpaw tree

2.7.1 Origin, Distribution and Domestication of Papaya

C.papaya is believed to have originated in Eastern Central America's plains, from Mexico to Panama (Basalingappa, 2010). Some suggest South America as its source, while others claim Central America as its center of origin (Basalingappa, 2010). It is generally grown between 32o North and South in the world's tropical and neo-tropical areas. Although papaya is thought to have been grown by early civilizations; there are no records available before Columbus's arrival in America (Basalingappa, 2010). Papaya plant is cultivated in tropical and subtropical nations, comprising 57 counties such as India, Brazil, Indonesia, Mexico and Nigeria. India is the biggest producer of papaya among these nations (Basalingappa, 2010). During the Spanish exploration of the 16th century, its seeds were distributed to the Caribbean and South East Asia, from where it spread rapidly to India, the Pacific and Africa (Basalingappa, 2010).

2.7.2 Common names

Some common names of *C.papaya* according to Orwa, *et al*., 2009 are:

Arabic (fafay, babaya), Bengali (pappaiya, papeya), Burmese (thimbaw), Creole (papayer, papaye), English (bisexual pawpaw, pawpaw tree, melon tree, papaya), Filipino (papaya, lapaya, kapaya), German (papaya, melon enbraum), Hindi(papaya, papeeta), Indonesian (gedang, papaya), Javanese (kates), Khmer(lhong, doeum lahong), Lao (Sino-Tibetan) (houng), Luganda (papaali), Malay (papaya, betek, ketalah, kepaya), Sinhala (pepol), Spanish (figueradel monte, fruta bomba, papaya, papaita, lechosa), Swahili (papai), Tamil(pappali, pappayi), Thai (ma kuai thet, malakor, loko), Tigrigna (papayo), Vietnamese (du du).

2.7.3 Taxonomy

C.papaya is a sole specie in the genus *Carica* of the plant family Caricaceae which is widely cultivated for its consumption.

Table 1: Taxonomy of *C. papaya* (Carvalho, *et al.*, 2013)

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Phylum	Streptophyta
Division	Magnoliophyta
Sub-division	Spermatophyta
Class	Magnoliopsida
Sub-class	Dilleniidar
Order	Brassicales
Family	Caricaceae
Genus	Carica
Species	*Carica papaya*

Table 2: Chemical composition of various parts of the Papaya (Fauziya and Krishnamurthy, 2013)

Part	Constituents
Fruits	Protein, fat, carbohydrates, minerals, vitamins, volatile compound, alkaloids, glycosides, calcium, phosphorus, iron, amino acids, citric and malic acids (green fruits).
Juice	N-butyric, n-hexanoic and n-octanoic acid, lipid, myristic, palmitic, stearic linoleic, linolenic acid and oleic acid
Seed	Fatty acid, crude protein, crude fibres, papaya oil, carpaine, benzyl isothiocyanate, benzylthiourea, β-sitosterol, caricin and enzyme myrosin.
Root	Caproside and enzyme myrosine
Leaves	Alkaloids carpain, pseudocarpain and dehydrocarpain 1,2, choline, caproside, vitamin C and E.

Bark	β-sitosterol, glucose, fructose, galactose and xylitol.
Latex	Proteolytic enzyme papain, chemopapain, glutamine cyclotransferase, chymopapain A,B,C, peptidase A and B, lysosome.

2.8 Pharmacological uses and activity of different parts of *C. papaya*

Whole *C.papaya* has a unique pharmacological uses: (Aravind, *et. al.,* 2013).

2.8.1 Leaves

Papaya leaf has many advantages. The young papaya leaves are cooked and consumed like spinach in some areas of Asia. (Aravind, *et. al.,* 2013).

a. Dengue fever

Study reports on 70 patients with papaya leaf juice showed enhanced white blood cells and platelets, standardized coagulation and repair of liver tissue (Aravind, *et. al.,* 2013).

b. Cancer Cell Growth Inhibition

Recent study on the extract of papaya leaf tea has shown inhibition of cancer cell development. It seems to increase the development of important signaling molecules called cytokines of the type Th1 that assist control the immune system (Aravind, *et al.,* 2013).

c. Antimalarial and Anti-plasmodial Activities

Papaya leaves are transformed into tea as a malaria therapy. Some plant preparations have recorded antimalarial and antiplasmodial operations, but the mechanism is not understood and is not scientifically proven (Aravind, *et. al.,* 2013).

d. Facilitate Digestion

The leaves of papaya crops contain chemical karpain compounds, a substance that kills microorganisms that often interfere with digestive function (Aravind, *et al.,* 2013).

Other uses of Papaya Leaves include:

- Acne medicine
- Increase appetite
- Ease menstrual pain
- Meat tenderizer
- Relieve nausea (Aravind, *et al.,* 2013)

2.8.2 Seeds

The papaya's black seeds are edible with a strong, spicy flavour. Sometimes they are ground and used as a replacement for black pepper (Aravind, *et al.,* 2013).

a. Nephro-Protective Activity

Nephro-protective activity was noted in dose-related way in Wistar rats. Urine and creatinine concentration were assessed (Aravind, *et al.,* 2013).

b. More Potency

- The seeds of papaya are very pungent and peppery, making them nearly unpalatable. The seeds, however, appear to have more powerful medicinal values than the flesh. (Aravind, *et al.,* 2013). Papaya seeds are antibacterial in nature and are efficient against infections with *E.coli,* Salmonella and Staphylococcus. Papaya seeds may protect the kidneys from toxin induced kidney failure.

- Papaya seeds can eliminate intestinal parasites.
- Papaya seeds help detoxify the liver
- Skin irritant to lower fever
- Cure for piles and typhoid
- Anti-helminthic and anti-amoebic properties (Aravind, *et al.*, 2013).

c. Anthelmintic Activity

The air-dried papaya seeds given as honey elixir have had significant effects on the parasites of the human intestine, without significant side effects. The presence of benzyl isothiocyanate in seeds results in the principal or sole anthelmintic activity. Papaya latex has anthelmintic effectiveness in experimentally infected animals against *Heligmosomoides polygyrus*, which indicates its potential function as an anthelmintic against mammalian host's powerful intestinal nematodes. It also has anthelmintic activity in cows against natural *Ascaris suum* disease and has been discovered to be 100% efficient at the 8g / kg body weight dose. Papaya crop extracts have a dose-dependent important impact on *Trichostrongylus colubriformis* ' egg, infective larvae, and adult worms. Papaya alcoholic extracts showed potential anti-parasitic action in vitro that impacts eggs, infectious larvae and adult *Haemonchus contorts* (Krishna, *et al.*, 2008).

d. Anti-amoebic Activity

The cold macerated water extract of mature papaya plants has demonstrated anti-amoebial activity against *Entamoeba histolytica* (Krishna, *et al.*, 2008)

e. Effect on smooth muscles

Ethanol extract of 0.1-6.4mg / ml papaya seeds showed concentration-dependent Jejuna contraction inhibition and was discovered to be considerably irreversible. As a result, seed extract is capable of weakening isolated rabbit jejunum's contractile capacity. Papaya seed

pentane extract has shown relaxation action on dog carotid artery strips pre-contracted with Phenylephrine. These are reported to be cytotoxic at the greater concentration due to the increased permeability of the membrane to calcium. A crude unripe fruit extract of ethanol generates a substantial depression of mean arterial pressure, but in the hypertensive rats the extract has about 28 percent more depression action than hydralazine. Papaya fruit juice is likely to contain anti-hypertensive agent(s), which primarily exhibits alpha adrenoreceptor activity. Extracts of papaya leaves had a relaxing impact of over 50 percent on aortic ring preparations (Krishna, *et al.,* 2008).

f. Male antifertility

Seed extract showed pronounced pituitary gonadotrophic hypertrophy and hyperplasia. While the male rats treated with seed extract disclosed gradual degeneration of germ, sertoli, and leydig cells as well as germ epithelium, confirming their antifertility activity. Aqueous extract of papaya plants, 3 weeks after the start of administration, showed that the lumina of the seminiferous tubules in experimental animals was more prominent and empty with no proof of sperm and spermatozoa. The chromatographic benzene fraction of the seed chloroform extract has reversible male contraceptive potential and the effect appears to be mediated through the testis and can be rendered directly on the sperm without adverse toxicity. Another research found sperm motility inhibition owing to other epididymal factors rather than the subcellular properties of testis and epididymis (Krishna, *et al.,* 2008).

2.8.3 Roots

Papaya root juice is used in some Asian nations to alleviate urinary disorders. Papaya leaf is smoked by asthmatic people when dried and healed like a cigar. To expel or kill intestinal worms, an infusion of new papaya leaves is used. Also used to cure colic or cramp is fresh young

papaya. A decoction created by boiling the outside of the papaya tree's roots is used to heal dyspepsia (Aravind, *et al.,* 2013).

a. Diuretic

When administered orally at a dose of 10 mg / kg to rats, aqueous root extract of papaya generates important increases in urine production and demonstrates comparable urinary electrolyte excretion profiles to that of hydrochlorothiazide (Krishna, *et al.,* 2008).

b. Female antifertility

The distinctive soft periodic pattern of ordinary epithelium appears to have altered in locations by haphazardly focused cell groups and microvill loss, whereas seed aqueous extract has shown abortive characteristics on female Sprague Dawley rats and the petroleum ether, alcoholic and aqueous extracts inhibit ovulation in rabbits. There was no anti-zygotic, anti-implantation, earlyabortion or antifertility activity in the papaya seed extracts. There may be no important risk of normal consumption of ripe papaya during pregnancy. However, unripe or semi-repeated papaya (which includes elevated latex concentration resulting in marked uterine contractions) may be dangerous during pregnancy (Krishna, *et al.,* 2008).

2.8.4 Latex

An unripe papaya's milky sap includes papain and chymopapain. In patients with documented herniated lumbar intervertebral disks, Chymopapain was approved for intradiscal injection and had not reacted to "conservative treatment." The latex also discovered vitamins and traces of an alkaloid called Carpaine. The fruit seeds, in addition to natural oils, also contain carbohydrates, carpasemine, benzyl senevol, and glucoside. Papain is also used as a meat tenderizer and in the manufacturing of chewing gums to treat commercial beer, degum natural silk. Cosmetically, it is used in a number of face-lifting operations and shampoos. Capaine slows the heart in animals, thereby reducing blood pressure (Aravind, *et al.*, 2013).

Papain has an impact of anticoagulant. Extract injection into a dog improves threefold prothrombin and coagulation. It is also asserted that in chronic injuries, burns and ulcers, the enzyme eliminates necrotic tissues. In the brewery sector, the food industry and the textile industry, as stated before, crude papain is also of commercial significance. Capaine slows the heart in animals, thereby reducing blood pressure. Higher doses can generate vasoconstriction, however, and anthelmintic activities are reported to occur in the alkaloid (Aravind, *et. al.,* 2013).

a. Antifungal Activity:

The papaya and fluconazole latex has synergistic intervention to prevent the development of Candida albicans. This synergistic impact leads to partial degradation of the cell wall (as stated by observations of transmission electron microscopy). Latex alone has a static effect. During the exponential growth stage, albicans were added to a crop and about 60 percent were attained. This fungal impact results from the degradation of the cell wall owing to the absence of polysaccharides in the outermost layers of the fungal cell wall and the release of cell debris into the culture medium (Krishna, *et al.,* 2008).

b. Histaminergic

Crude latex triggers contraction of isolated guinea pig ileum strips that are mediated by an H1 receptor and are dependent on extracellular Ca2 + influx. Papaya flower pollen can cause IgE-mediated allergy to the respiratory system. RAST inhibition has shown the presence of prevalent allergens among papaya flower pollen, fruit and papain (Krishna, *et al.,* 2008).

2.8.5 Fruit

Papaya fruit is a wealthy source of nutrients like carotenoid provitamin, vitamin C, vitamin B, lycopene, nutritional minerals, and dietary fiber. Danielone is a papaya fruit phytoalexin. This

compound showed elevated antifungal activity against *gloesporioides of Colletotrichum* (Aravind, *et. al.,* 2013).

a. Hepatoprotective

Fruit ethanol and aqueous extracts have notable hepatoprotective activity against the hepatotoxicity caused by CCl_4. But the mechanism of hepatoprotection and the active values responsible for this plant's hepatoprotective activity are not yet understood (Krishna, *et al.,* 2008).

b. Laxative

Ripe papaya fruit is laxative to ensure periodic motion of the intestine (Aravind, *et al.,* 2013)

c. Indigestion

The milky juice that is sucked from the mature green fruit while still in the tree includes a papain enzyme. People use this to prepare various indigestion remedies (Aravind, *et al.*, 2013).

d. Heart Attack or Stroke

To convert homocysteine into amino acids such as cysteine or methionine, the folic acid found in papayas is required. If unconverted, homocysteine can directly harm the walls of the blood vessel, an important risk factor for a heart attack or stroke is regarded (Aravind, *et al.*, 2013).

2.8.6 Peel

In cosmetics, papaya peel is often used. In many home remedies, the papaya peel can also be used (Aravind, *et al.,* 2013).

a. Sunscreen and Soothing Slave

Vitamin A presence helps restore and reconstruct damaged skin. Used as a skin lightening agent for papaya peel. It can function as soothing and moisturizing the skin when peeling blended with honey and applied (Aravind, *et al.,* 2013)

b. Fight Dandruff

To combat dandruff, the papaya vinegar with lemon juice can be added to the scalp 20 minutes before shampooing.

c. Muscle Relaxant

Adding papaya oil and vinegar to bath water can be nourishing, refreshing and soothing, along with essential oils such as lavender, orange and rosemary, and can function as a pain reliever and relaxing muscle (Aravind, *et al.*, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

Fresh pawpaw leaves (*C.papaya*) was used for this research.

3.1.1 Sample Collection and Preparation

The pawpaw leaves were collected from an individual farm at Amofe Adetoye Street, Ayobo Ipaja, and Lagos state in January 2019. The leaves were separated from the stem and air-dried to remove moisture present in them. They were then ground by means of mechanical blender into powdery form. The powdered pawpaw leaves was weighed and stored in air-tight jar and kept in a refrigerator. Seventy grams (70 g) of the powder was weighed into three (3) jars and was soaked in 70%methanol (ratio 8:1 w/v) with intermittent shaking at room temperature for 72hours.

The solution was then filtered using muslin cloth. Subsequently, the solvent was evaporated under reduced pressure at a controlled temperature using a rotary evaporator. The concentrates were kept in the oven (40°C) for complete dryness of the methanol extracts and were then stored in a refrigerator at 4°C. The percentage yield of the extracts was calculated.

3.2 Qualitative Phytochemical Screening

The methanolic extract was tested for the presence of bioactive compounds using standard methods as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973) with slight modification.

3.2.1 Test for saponin (Froth test)

0.5g of extract was diluted with distilled water to 20ml and was shaken in a graduated cylinder for 5mins. Formation of foam indicated the presence of saponin.

3.2.2 Test for terpenoids (Salkowski's test)

0.5g of extract was dissolved in 5mls of distilled water. 2ml of chloroform was added and 3ml of conc. H_2SO_4 was carefully added to form a layer. The appearance of reddish brown coloration at the interphase indicated the presence of terpenoids.

3.2.3 Test for phenol (Ferric chloride test)

0.5g of extract was dissolved in 5mls of distilled water and 4 drops of ferric chloride ($FeCl_3$) solution was added. The formation of bluish black color indicated the presence of phenol.

3.2.4 Test for flavonoid (Alkaline test)

0.5g of extract was dissolved in 5mls of distilled water and few drops of 10% sodium hydroxide (NaOH) solution were added. The formation of intense yellow color indicated the presence of flavonoid.

3.2.5 Test for alkaloids (Mayer's test)

0.5g of the crude extract was dissolved in 5mls of distilled water. 2ml of 1% hydrochloride (HCl) was added and heated gently. 3mls of Mayer's reagent was added to the mixture. Turbidity of the resulting precipitate indicated the presence of alkaloids.

3.2.6 Test for carbohydrates (Molisch's test)

0.5g of extract was dissolved in 5mls of distilled water. 2mls of Molisch reagent was added and the mixture was shaken properly. 2ml of conc. sulphuric (H_2SO_4) was poured carefully along the

side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

3.2.7 Test for glycosides (Borntrager's test)

0.5g of extract was dissolved in 5mls of distilled water. 3mls of chloroform was added and the mixture was shaken. The chloroform layer was separated and 2ml 0f 10% ammonia solution was added. The appearance of pink color indicated the presence of glycosides.

3.2.8 Test for tannin

0.5g of extract was dissolved in 5mls of distilled water and 2mls of 2% $FeCl_3$ solution was added. The formation of blue-green coloration indicated the presence of tannin.

3.2.9 Test for protein (Ninhydrin test)

0.5g of extract was dissolved in 5mls of distilled water. 2mls of 0.2%ninhydrin reagent was added and the mixture was boiled for 5mins. The formation of violet/blue color indicated the presence of amino acids.

3.2.10 Test for phytosterol (Libermann-Burchard's test)

0.5g of extract was dissolved in 5mls of distilled water. 2 drops of conc. H_2SO_4 was added slowly along the side of the test tube. Change in color (violet to blue) indicated the presence of steroids.

3.2.11 Test for polyphenol

0.5g of extract was dissolved in 5mls of distilled water. 1ml of 2% $FeCl_3$ solution and 1ml of 1% potassium ferricyanide solution were added. The formation of green-blue color indicated the presence of polyphenol.

3.2.12 Test for anthraquinone

0.5g of crude extract was dissolved in 5mls of distilled water. 10ml of conc. H_2SO_4 was added and filtered. 5ml of chloroform was added to the filtrate and the chloroform layer was pipette into another test tube. 1ml of ammonia was added. The formation of pink-red color indicated the presence of anthraquinone.

3.2.13 Test for fat and oil (Spot test)

Small quantity of the extract was pressed between two filter papers. The appearance of oil stain on the paper indicated the presence of fixed oil.

3.3 Experimental animals

Thirty (30) male Wistar rats weighing (115-230g) were randomly arranged in well ventilated cages under controlled conditions of 12hours light/dark cycle at the Animal house of the Department of Biological Sciences, Mountain Top University, Nigeria. All the rats were left to acclimatize for seven (7) days. They were maintained on standard feed and water *ad libitum*. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals based on the guidelines of the Institutional Animal Ethics Committee (IAEC).

3.4 Induction of diabetes

Animals of Groups II-V were weighed, and their fasting glucose levels were determined before inducing diabetes. A single intraperitoneal injection of streptozotocin (55 mg/kg bw) dissolved in 0.1M citrate buffer (pH 4.5) for injection was administered after fasting. Control animals (group I) received distilled water as placebo while group II-V received 5% glucose solution for the next 12hours to overcome STZ-induced hypoglycemia. Animals were checked for successful

induction of diabetes after 72 hours (post induction). Hyperglycemia was confirmed three days after injection and animals with blood glucose > 200 mg/dL were classified as diabetic.

3.5 Experimental design

Group I– Normal Control (given distilled water)

Group II – STZ-Induced Diabetic rats given distilled water

Group III – Diabetic rats administered with 100mg/kg body weight of C. papaya leaf methanol extract.

Group IV – Diabetic rats administered with 200mg/kg body weight of C. papaya leaf methanol extract.

Group V – Diabetic rats administered with metformin (standard drug).

The treatments were administered daily for 2 weeks with the leaf methanol extract. Blood samples for Fasting blood glucose (FBG) determination were collected via a slight incision on the lateral tail vein using a scalpel blade. The measurements were taken in duplicate to ensure consistency in the glucometer readings. At the end of administration, the rats were sacrificed under light anesthesia (by placing the animal in chloroform fume chamber for 10 seconds). Blood samples were collected through ocular and cardiac puncture into Lithium heparin (EDTA) bottles and centrifuged at 2500rpm for 15mins and the plasma stored for biochemical assays.

3.6 Tissue preparation

The liver was excised and washed in 0.9% NaCl (PBS) and homogenized at 1500rpm for 10mins to obtain the supernatant for assays.

3.7 Determination of blood glucose level

Using Randox kit, the glucose level was investigated. Test tubes were labeled according to group and identity given to the experimental animals, alongside one test tube for blank and one test tube for standard.

Standard reagent (20µl) was pipetted into a test tube and 200µl of working reagent was added. This was repeated into corresponding test tubes. All the test tubes were incubated at 37°C for 10minutes and was read at a wavelength of 500nm against the blank that contained only working reagent. Concentration of Plasma glucose was obtained by calculating:

(Absorbance of sample/ absorbance of standard) X concentration of standard (103mg/dL).

3.8 Clotting and bleeding time

The clotting time was determined using Ivy's method as reported by Ibu and Adeniyi (1989). A drop of blood from the tail of each rat was placed on a clean glass slide and a stopwatch began at the same time. A pin was passed across once every 15seconds. As soon as a thread of fibrin was noticed, the stopwatch was stopped and the time was recorded as the clotting time for the rat.

The bleeding time was determined using Shrivasta and Das (1987) as reported by Raof *et al.* (2013). The rat's tail was cut with a scalpel 1-2cm proximal from the end. A stop watch began immediately to observe till bleeding stop. Spots were made with the bleeding tail on a filter paper every 15seconds until bleeding stops. The time was recorded as the bleeding time of the rat.

3.9 Assays for lipid profile

Lipid profile which includes; Total Cholesterol (TC), Triacylglycerol (TAG), High-density Lipoprotein-Cholesterol (HDL-C) was determined using standard laboratory kit from Randox laboratories, UK.

According to Feriedewaki *et al.*, (1972), estimation of Very Low density Lipoprotein-Cholesterol (VLDL-C) and Low density Lipoprotein-Cholesterol (LDL-C) was done by:

VLDL-C= TAG/5

LDL-C= TC- (VLDL-C+ HDL-C).

3.10 Waste disposal

The experimental wastes were incinerated and the experimental animals were buried.

3.11 Statistical analysis

Results obtained were presented as mean ±SD. Variation within a set of data was analyzed by one-way analysis of variance (ANOVA) using the Graph Pad Prism Software (GPPS) 8.0. Values of $p<0.05$ were taken as statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening

The phytochemical screening of *C. papaya* leaf methanol extract showed the presence of alkaloids, glycosides, flavonoids, phenol, anthraquinone, tannin, steroids, etc.

Table 3: Qualitative phytochemical constituents of *C. papaya* leaf methanol extract

PHYTOCHEMICAL TEST	RESULTS
ALKALOIDS	++
CARBOHYDRATES	+
GLYCOSIDES	+
SAPONIN	++
TEREPONOIDS	++
POLYPHENOL	+
FLAVONOIDS	++
TANNIS	++
PROTEIN	++
PHYTOSTEROL	++
ANTHRAQUINONE	+
FAT AND OIL	+
PHENOL	++

+ Represent presents, - represents No activity;

4.2 Effects of administration of methanol extract of *C. papaya* on plasma glucose concentration of STZ induced diabetic rats.

From figure 4, there was a significant increase ($p<0.05$) in plasma glucose concentration of diabetic control compared to the normal control. Administration of the plant extract at both concentrations (100mg/dL and 200mg/dL) significantly reduced the plasma blood glucose in diabetic rats. Similar result was recorded with the standard drug (Metformin).

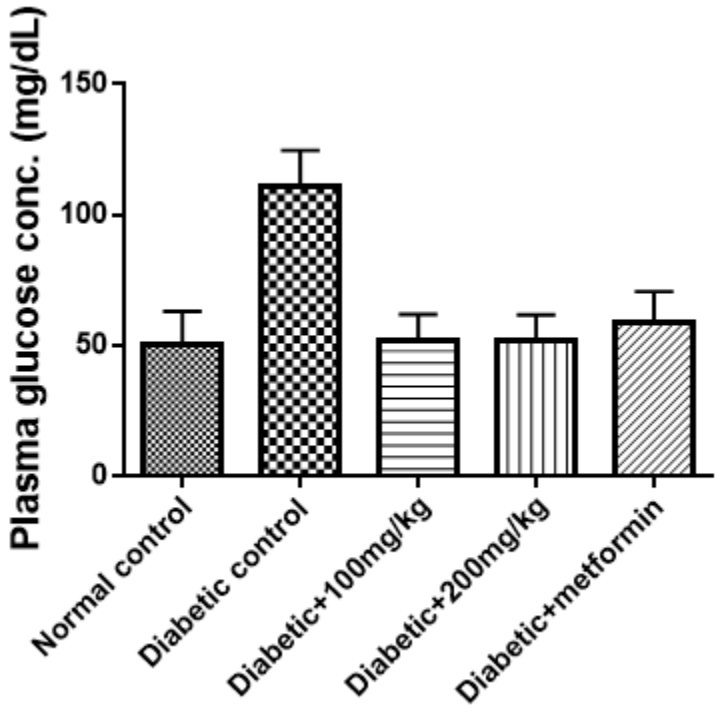

Figure 4: Plasma glucose concentration of Normal and diabetic rats.

Extract was dissolved in distilled water (vehicle); n=4

4.3 Effects of administration of methanol extract of *C. papaya* leaves on plasma Total cholesterol of STZ induced diabetic rats.

The results showed a significant increase ($P < 0.001$) in plasma cholesterol concentration of diabetic control rats compared to the normal control rats, whereas treatment with the plant extract and standard drug significantly reduced the cholesterol concentration compared to diabetic control group.

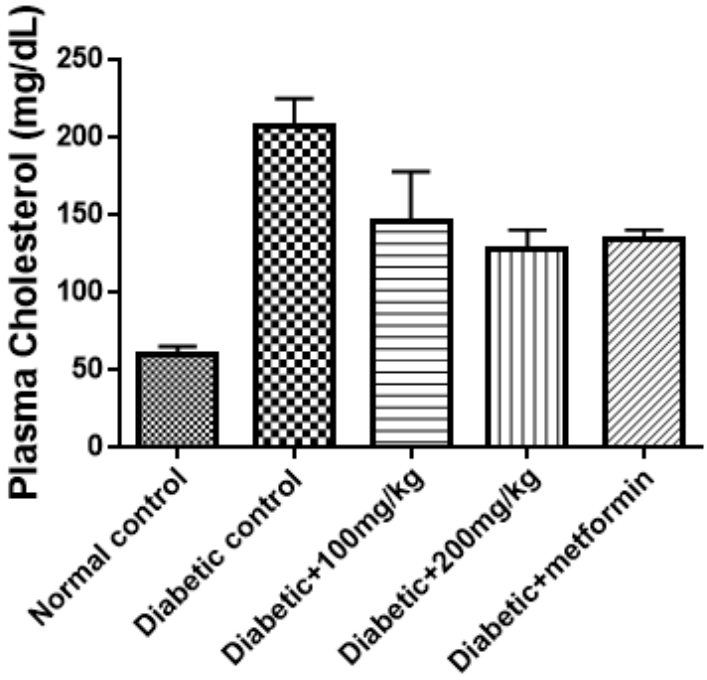

Figure 5: Plasma Total cholesterol concentrations of Normal and Diabetic rats

Extract was dissolved in distilled water (vehicle); n=4

4.4 Effects of administration of methanol extract of *C. papaya* leaves on plasma triglycerides of STZ induced diabetic rats.

There was a significant increase ($P < 0.05$) in plasma triglycerides concentration of diabetic control group compared to the normal control group. There was significant decrease in the plant extract treated groups compared to the diabetic control, but the highest reduction was observed with 200 mg/dL plant extract intervention.

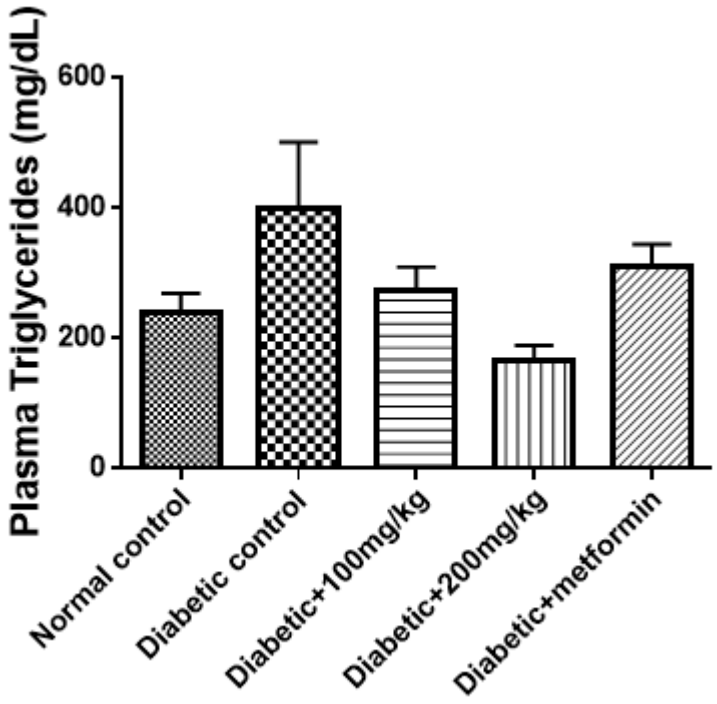

Figure 6: Plasma Triglycerides concentrations of Normal and diabetic rats

Extract was dissolved in distilled water (vehicle); n=4

4.5 Effects of administration of methanol extract of *C. papaya* leaves on plasma HDL-C of STZ induced diabetic rats.

There was a significant decrease ($P < 0.05$) of plasma HDL-C concentration of the diabetic control rats compared to the normal control. Significant increase were recorded in diabetic rats treated with plant extract and metformin compared with the diabetic control rats.

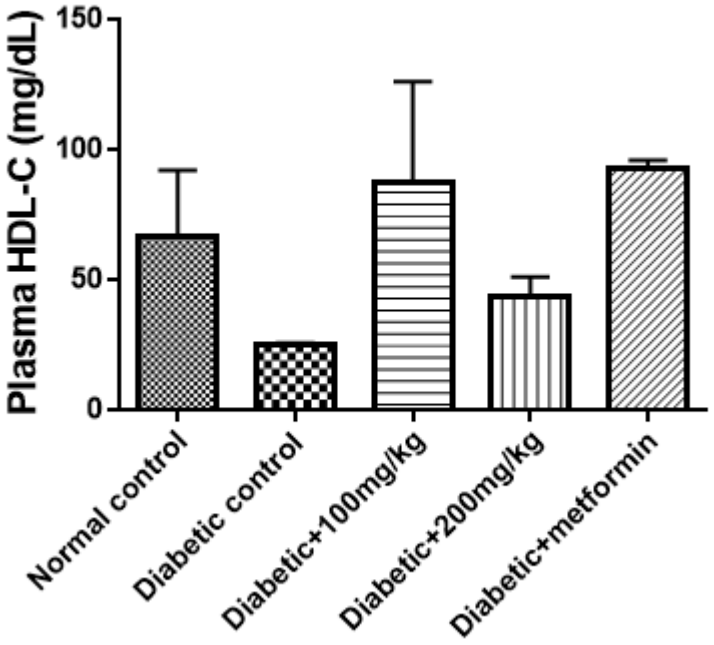

Figure 7: Plasma HDL-C concentrations of Normal and diabetic rats

Extract was dissolved in distilled water (vehicle); n=4

4.6 Effects of administration of methanol extract of *C. papaya* leaves on plasma VLDL-C of STZ induced diabetic rats.

The plasma VLDL-C was significantly ($P < 0.05$) increased in the diabetic control compared to the normal control. The intervention of the plant extract reduced the plasma VLDL-C of the diabetic rats.

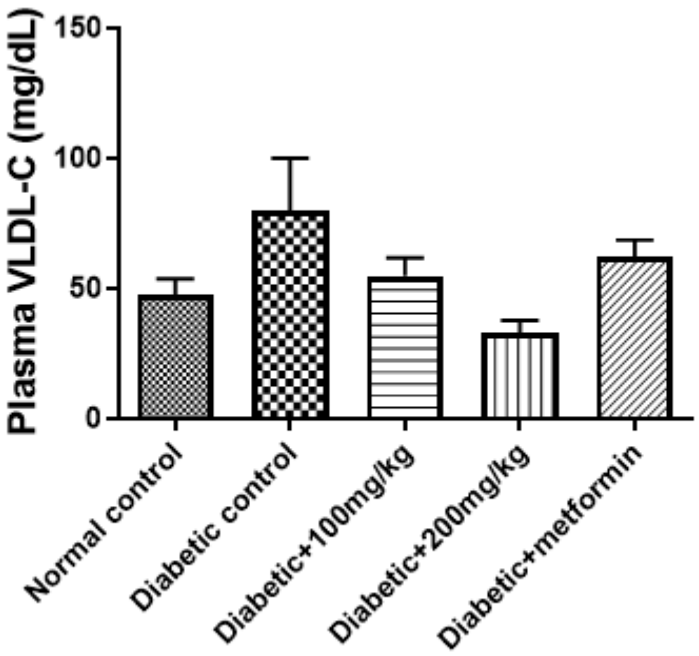

Figure 8: Plasma VLDL-C concentrations of Normal and diabetic rats

Extract was dissolved in distilled water (vehicle); n=4

4.7 Effects of administration of methanol extract of *C. papaya* leaves on plasma LDL-C of STZ induced diabetic rats.

There was no significant difference between the control group and the intervention of the plant extract, however there was a significant decrease with the standard drug (metformin) compared to other groups.

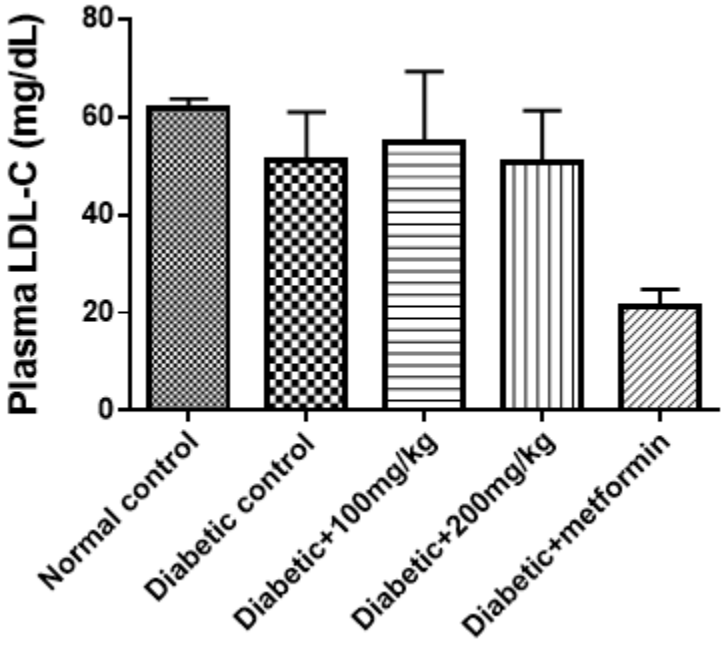

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Figure 9: Plasma LDL-C concentrations of Normal and diabetic rats.

Extract was dissolved in distilled water (vehicle); n=4

4.8 Effects of administration of methanol extract of *C. papaya* leaves on bleeding time of STZ induced diabetic rats.

The bleeding time was reduced in diabetic control rats compared to the normal rats and administration of plant extract to the diabetic rats further reduced the bleeding time. There were no differences in results recorded with the plant extract at both concentrations and metformin.

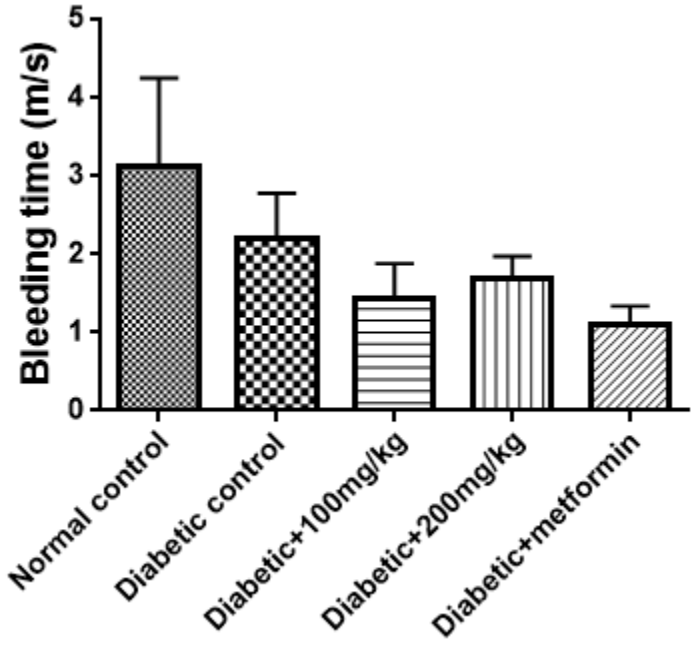

Figure 10: Bleeding time of Normal control and diabetic rats.

Extract was dissolved in distilled water (vehicle); n=4

4.9 Effects of administration of methanol extract of *C. papaya* leaves on blood clotting time of STZ induced diabetic rats.

There was no significant difference between the normal control and the diabetic control, and diabetic rats given 100mg/kg body weight of plant extract. However, the clotting time was significantly reduced in diabetic groups given 100mg/kg body weight of plant extract and metformin.

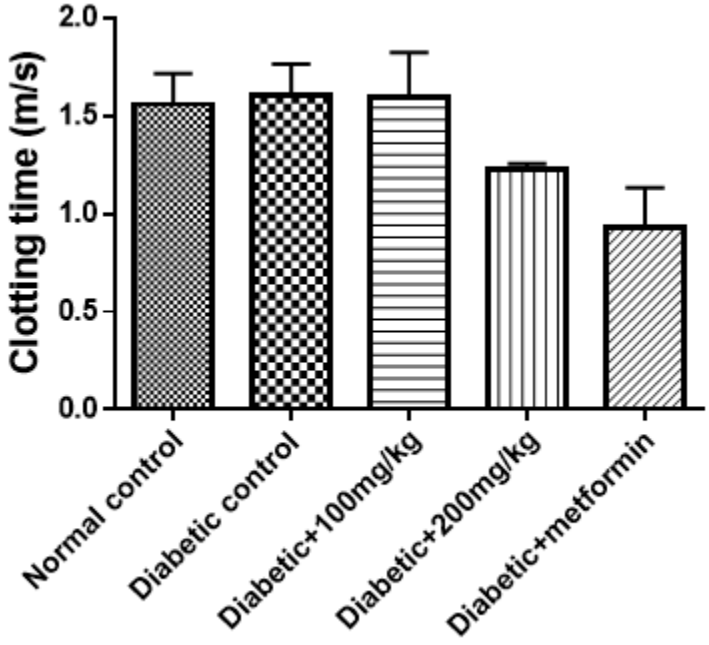

Figure 11: Blood Clotting times of normal and diabetic rats.

Extract was dissolved in distilled water (vehicle) n=4

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Presently, herbal products are being used as a source in medicine. The medicinal properties of plants have been part of ancient knowledge, and modern medicine benefits from them. In this sense, phytochemicals and their derivatives have been an extraordinary source of lead compounds for therapeutics and drug development. In this study, the effect of *C. papaya* leaf methanol extract on lipid profile and blood clotting of diabetic rats was investigated.

This study showed significantly increased blood glucose levels in diabetic rats compared to normal rats. Administration of *C. papaya* leaf methanol extract significantly ($p<0.05$) decreased the plasma glucose concentrations in diabetic treated rats. Similar result was recorded with metformin. Juarez- Rojop, *et al.* (2012) similarly reported that the *C. papaya* leaf aqueous extract significantly diminished blood glucose levels in diabetic rats. Such effect may be explained in part by either a decrease in the rate of intestinal glucose absorption (Hamden, *et al.*, 2011) or an increase in peripheral glucose utilization (Porchezhian, *et al.*, 2000).

This study also showed that *C. papaya* leaf methanol extract significantly decreased the plasma triglycerides, total cholesterol, LDL-C and VLDL-C level in diabetic rats which concentrations were elevated compared to normal control rats. Juarez- Rojop, *et al.* (2012) also demonstrated that triacylglycerol and cholesterol levels were decreased in diabetic rats with the administration of *C. papaya* aqueous extract. Diabetes-related hyperlipidemia may lead from an accelerated biosynthesis of hepatic triglycerides. The plasma cholesterol, TAG, LDL-C and VLDL-C lowering effect of *C. papaya* may be attributed to its ability to ameliorate hyperlipidemia by decreasing the

activities of cholesterol biosynthesis enzymes and/or lowering the level of lipolysis which are under the control of insulin (Sharma, *et al.,* 2003).

HDL-C in this present study was decreased significantly ($P<0.05$) in diabetic control rats compared to the normal rats. Significant increase were recorded in diabetic rats treated with plant extract and metformin compared with the diabetic control rats. This is as a result of the hypolipidemic effect present in *C. papaya*. This hypolipidemic effect may cause a rise in HDL-C level as this is a good cholesterol for the prevention of cardiovascular diseases (Debidas, *et al.*, 2005). Similar increase in HDL-C concentration was reported by Oyewole, *et al.* (2013) on the serum cholesterol lowering effect of *Ficus exasperata* which may be attributed to its ability to increase the excretion of cholesterol. These results suggest that *C. papaya* can be used as anti-atherogenic agent for the management of atherosclerosis.

The present study showed a decrease in bleeding time and clotting time in diabetic control rats compared to the normal rats. *C. papaya* leaf methanol extract further reduced the clotting time and the bleeding time of the diabetic rats. The observed marked reduction in blood clotting parameters tested may be due to the hyper-coagulation state reported in diabetic mellitus. Ability of *C. papaya* leaves to further decrease the bleeding and clotting times showed the procoagulant tendency of the plant which may be attributed to reported anti-platelet activity of *C. papaya* (Oyewole, *et al.,* 2013).

The result of the qualitative phytochemical screening of *C. papaya* leaves showed that the methanol extract contains a great proportion of steroids, tannins, anthraquinones, alkaloids and other bioactive compounds. Some of this phytochemicals are responsible for the hypoglycemic, hypolipidemic and proagulation effect in diabetic rat. Juarez-Rojo, *et al.* (2012) also reported the

presence of steroids, tannin, glycosides, anthraquinone etc. in phytochemical screening of aqueous extract and hexane fraction of *C. papaya* leaves.

5.2 Conclusion

This study showed and confirmed that *C. papaya* leaf methanol extract possess hypoglycemia, hypolipidemia and procoagulant activities. Therefore, *C. papaya* leaves may be a source of novel anti-hyperglycemic, hypolipidemic and procoagulant agents in pharmaceutical drug development. Findings in this study indicate that *C. papaya* leaf extract may be beneficial to diabetic patients and helpful in the prevention of diabetic complications by dyslipidemia improvement.

5.3 Recommendation

Further study is required on blood coagulation parameters to explain the mechanism by which *C. papaya* affects procoagulation effect and to elucidate the mechanism of hypolipidemia and hypoglycemia of *C. papaya* leaves.

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APPENDIX

Table 4: Effects of administration of *C. papaya* leaf methanol extract on plasma glucose concentration of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	50.00±13.13	111.0±13.67	52.00±9.79	52.00±9.721	58.75±11.81

Values are expressed as mean ± standard error of mean

Table 5: Effects of administration of *C. papaya* leaf methanol extract on plasma total cholesterol of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	59.71±5.25	207.5±17.24	145.8±31,89	127.8±12.35	133.9±6.16

Values are expressed as mean ± standard error of mean

Table 6: Effects of administration of *C. papaya* leaf methanol extract on plasma triglycerides of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	237.4±31.02	237.4±100.5	273.9±34.77	273.9±34.77	310.4±32.76

Values are expressed as mean ± standard error of mean

Table 7: Effects of administration of *C. papaya* leaf methanol extract on plasma HDL-C of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	66.73±25.44	25.10±0.7163	87.74±38.47	43.86±7.200	92.91±3.011

Values are expressed as mean ± standard error of mean

Table 8: Effects of administration of *C. papaya* leaf methanol extract on plasma LDL-C of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	61.80± 1.903	51.32± 9.683	54.76± 14.49	50.78± 10.53	21.12± 3.629

Values are expressed as mean ± standard error of mean

Table 9: Effects of administration of *C. papaya* leaf methanol extract on plasma VLDL-C of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	47.48±6.203	79.89±20.10	54.78±6.955	33.17±4.447	62.09±6.552

Values are expressed as mean ± standard error of mean

Table 10: Effects of administration of *C. papaya* leaf methanol extract on bleeding time of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	3.120±1.128	2.190±0.588	1.438±0.442	1.678±0.291	1.903±0.240

Values are expressed as mean ± standard error of mean

Table 11: Effects of administration of *C. papaya* leaf methanol extract on clotting time of normal and diabetic rats

	Group 1 (Normal control)	Group 2 (diabetic control)	Group 3 (Diabetic+ 100mg/kg)	Group 4 (Diabetic+ 200mg/kg)	Group5(Diabetic+metformin)
Concentration (g/dL)	1.560± 0.158	1.603± 0.1621	1.598± 0.2271	1.228± 0.02780	0.9275± 0.2062

Values are expressed as mean ± standard error of mean